

## **LISTING OF THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. **(Original)** - Electroporation device for the permeabilization of cells (C) contained in a substrate (12) comprising signal generating means (3) for generating a stimulating signal (S(t)) applied by means of electrodes (6,7) to the substrate (12) wherein an electric field (E(t)) permeabilizing the cells membranes is induced;

the device being characterized by comprising:

- means for measuring, calculating and monitoring (15,16,23) the instantaneous value of the ration (GT) of current (ie) flowing between said electrodes (6,7) and through the substrate (12) and the voltage (Vp) of the stimulating signal (S(t)) applied to the substrate (12) by means of said electrodes (6,7);

said device further comprising controlling means (100-170) for applying the stimulating signal in a controlled manner according to the waveform of an initial portion of the curve  $C_{GT}$  representing the ratio (GT) in successive instants after the beginning of the application of the stimulating signal (S(t)).

2. **(Original)** - Device as claimed in claim 1, wherein said controlling means (100-170) comprise timing means (110) for applying said stimulating signal for a predetermined period of time Td and analysing the initial portion of the waveform of curve  $C_{GT}$  to detect a minimum value of the curve  $C_{GT}$  within the interval  $t = 0$  and  $t = Td$ .

3. **(Currently Amended)** - Device as claimed in claim 1 ~~or 2~~, wherein said controlling means (100-170) calculate the slope of the waveform of curve  $C_{GT}$  after that a minimum in curve  $C_{GT}$  has been reached.

4. **(Currently Amended)** - Device as claimed in claim 1 ~~any of the preceding claims~~, wherein said controlling means (100-170) comprise hazard detecting means (120)

determining the instantaneous gradient (dG) of said ratio (GT) after a minimum has been reached in said curve  $C_{GT}$ ;

said controlling means further comprise first comparing means (130) for comparing the calculated instantaneous gradient dG with at least a reference value ( $dG_{ref}$ ) and selecting correcting means (140,145) for performing an urgent correction to the stimulating signal S(t) in order to avoid lesions, damages or irreversible alterations in said substrate (12).

**5. (Original)** - Device as claimed in claim 4, wherein said correcting means (140,145) decreases the voltage of the stimulating signal S(t) in order to prevent deterioration in the cells (C).

**6. (Currently Amended)** - Device as claimed in claim 1 ~~any of the preceding claims~~, wherein said controlling means (100-170) comprise slope determining means (150) calculating the average variation  $\Delta G$  of said ratio (GT) in a time interval that is successive to the instant  $T_m$  wherein a minimum in the curve ( $C_{GT}$ ) has been reached and that has a pre-determined time width;

said controlling means further comprising second comparing means (160) comparing the calculated average variation  $\Delta G$  of said ratio (GT) with a reference interval of  $\Delta G$  values.

**7. (Original)** - Device as claimed in claim 6, wherein said second comparing means (160) performs the following functions:

- if the calculated average variation  $\Delta G$  of said ratio (GT) falls within the reference interval ( $0 < \Delta G < \Delta G_{obb}$ ) continuing means (170) are selected;
- if the calculated average variation  $\Delta G$  of said ratio (GT) falls outside the reference interval and it is smaller than both limits delimiting the interval ( $\Delta G < 0 < \Delta G_{obb}$ ) adjusting means (180) are selected; and
- if the calculated average variation  $\Delta G$  of said ratio (GT) falls outside the reference interval and it is greater than both limits delimiting the interval ( $\Delta G > \Delta G_{obb} > 0$ ) correcting means (140) are selected.

8. **(Original)** - Device as claimed in claim 7, wherein said adjusting means (180) increase the voltage of the stimulating signal in order to increase the value of the electric field  $E(t)$  applied to the substrate (12); said adjusting means (180) subsequently selecting said means for calculating and monitoring (15,16,23) the instantaneous value of the said ratio (GT) and said controlling means.

9. **(Original)** - Device as claimed in claim 7, wherein said continuing means (170) increase the voltage of the stimulating signal to an objective voltage  $V_{opt}$  in order to increase the value of the electric field  $E(t)$  applied to the substrate (12) so that the value of said average variation  $\Delta G$  tends to an expected value  $\Delta G_{obb}$ .

10. **(Currently Amended)** - Device as claimed in claim 1 ~~any of the preceding claims~~, wherein said controlling means (100-170) detects (125) a minimum of said initial portion of said curve and determining (126) the time  $T_m$  at which the minimum is reached.

11. **(Original)** - Device as claimed in claim 10, wherein third comparing means (127) are provided to compare the detected  $T_m$  with threshold values  $T_{tmin}$  and  $T_{tmax}$ ;

said third comparing means (127) performing the following operations:

- if the detected  $T_m$  occurs before  $T_{tmin}$  ( $T_m < T_{tmin}$ ) then correcting means (140) are selected;
- if the detected  $T_m$  occurs after  $T_{tmax}$  ( $T_m > T_{tmax}$ ), then adjusting means (180) are selected; and
- if the detected  $T_m$  occurs between  $T_{tmin}$  and  $T_{tmax}$ , then continuing means (170) are selected.

12. **(Currently Amended)** - Use of a device as described in claim 1 ~~any of the preceding claims~~, to extract molecules from the living cells comprised in the substrate.

**13. (Currently Amended)** - Use of a device as described in claim 1 ~~any of the preceding claims~~, to introduce molecules into living cells.

**14. (Original)** - Use of the device as claimed in claim 13, wherein said molecules comprise one of the following:

- ◆ a DNA or a RNA molecule containing regulatory sequences and sequence coding for therapeutic genes or genes of interest for biomedical or biotechnological purposes;
  - ◆ an oligonucleotide, (ribo- or deoxyribo- nucleotide, single or double strand, including the SiRNA), whether natural (phosphodiester) or modified (inside the backbone of the oligonucleotide, such as phosphosulfates, or at the extremities, by addition of groups to protect the oligonucleotides from digestion of nucleases;
  - ◆ a protein or peptide, whether natural or genetically or chemically modified, extracted from natural sources or obtained by synthesis, or a molecule simulating the structure of a protein or peptide, whatever its structure;
  - ◆ a cytotoxic agent, in particular, the antibiotic bleomycin or the cisplatin;
  - ◆ a penicillin; and
- other pharmacological agents.